A new nanomedicine platform to deliver a carnitine palmitoyl-transferase 1 (CPT1) inhibitor into glioma cells and hypothalamic neurons

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INTRODUCTION

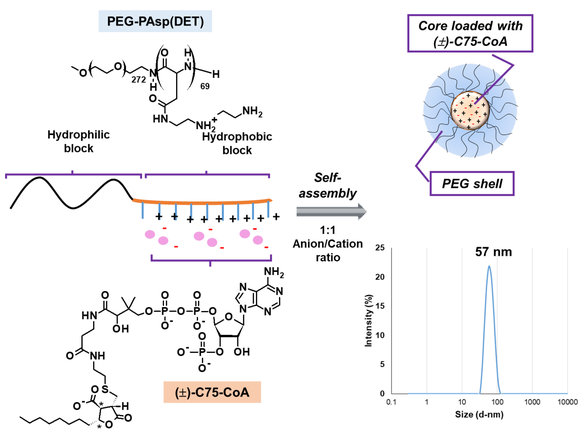
Obesity and glioblastoma multiforme (GB) are two unmet medical needs which are linked by cellular fatty acid (FA) metabolism. **Carnitine palmitoyl transferase 1 (CPT1)**, an enzyme involved in **fatty acid oxidation (FAO)** [1], is a viable target for both diseases. Inhibition of CPT1 in the hypothalamus contributes to reduced expression of orexigenic proteins and diminished food intake because of neuronal FA-CoA accumulation [2,3]. CPT1 is also crucial to the survival of GB cells, where it is overexpressed [4]. Its inhibition a possible strategy to suppress GB tumor growth [5].

OBJECTIVE

C75-CoA is a strong competitive inhibitor to CPT1 [6] . However, it is polar and negatively charged, having low cell membrane permeability, and therefore needing a delivery system [7] for intracellular transport. Thus, we aim **to deliver C75-CoA through a nanomedicine platform (poly-ion complex [PIC] micelle) into selected brain cells** for CPT1 targeting.

METHODS

(±)-C75-CoA and its enantio-pure forms (+)- and (-)-C75-CoA were prepared by a thiol-ene reaction between CoA and the corresponding stereoisomer of C75 at pH 8. The adduct was mixed with PEG-PAsp(DET) to form PIC micelles. Cytotoxicity, ATP levels, and cellular uptake were measured in both **U87MG human glioma cells** and **GT1-7 murine hypothalamic neurons**.



RESULTS

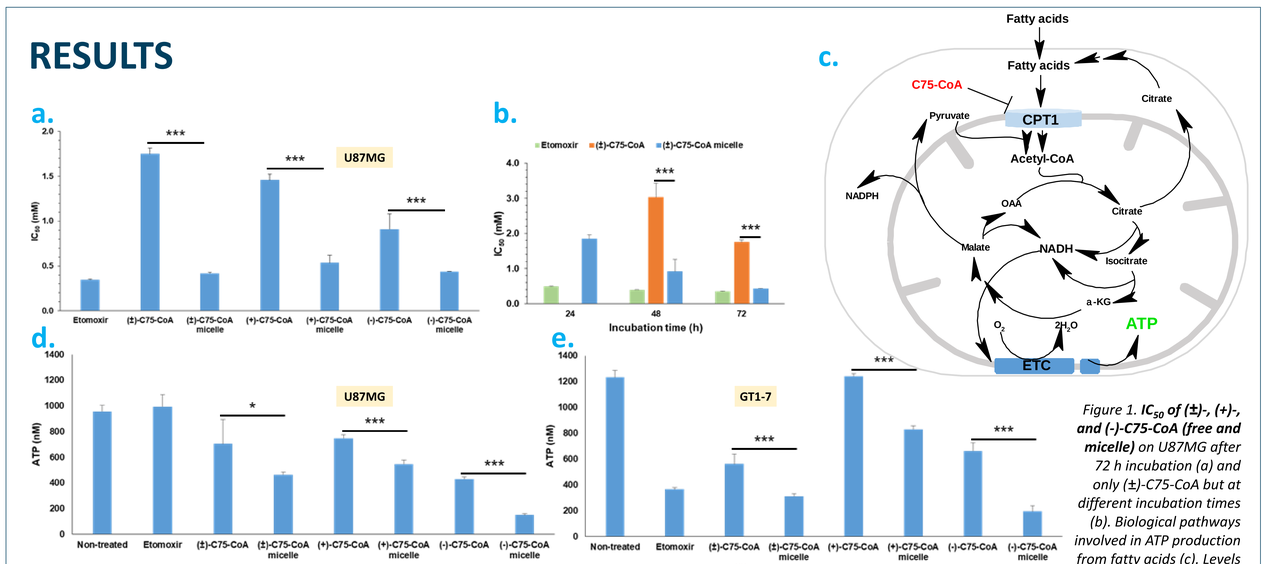
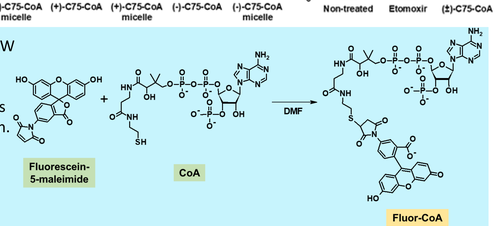


*Figure 1. **IC_{50} of (±)-, (+)-, and (-)-C75-CoA (free and micelle)** on U87MG after 72 h incubation (a) and only (±)-C75-CoA but at different incubation times (b). Biological pathways involved in ATP production from fatty acids (c). Levels of **ATP** produced by **U87MG (d)** and **GT1-7 (e)** after treatment with FAO inhibitors, normalized using cell viability values from Calcein AM staining (n=3, mean ± sd, student's t-test * p<0.05, ** p<0.01, *** p<0.001).*

Synthesis of **Fluor-CoA**: It has a MW close to C75-CoA, including the same anionic groups, imparting similar physicochemical properties + additional fluorescence emission. It was used as model compound and was encapsulated into PIC micelle (size: **47 nm**) to track cell internalization.



Delivery through PIC micelles led to higher cellular concentration of the cargo, which boosted biological activity

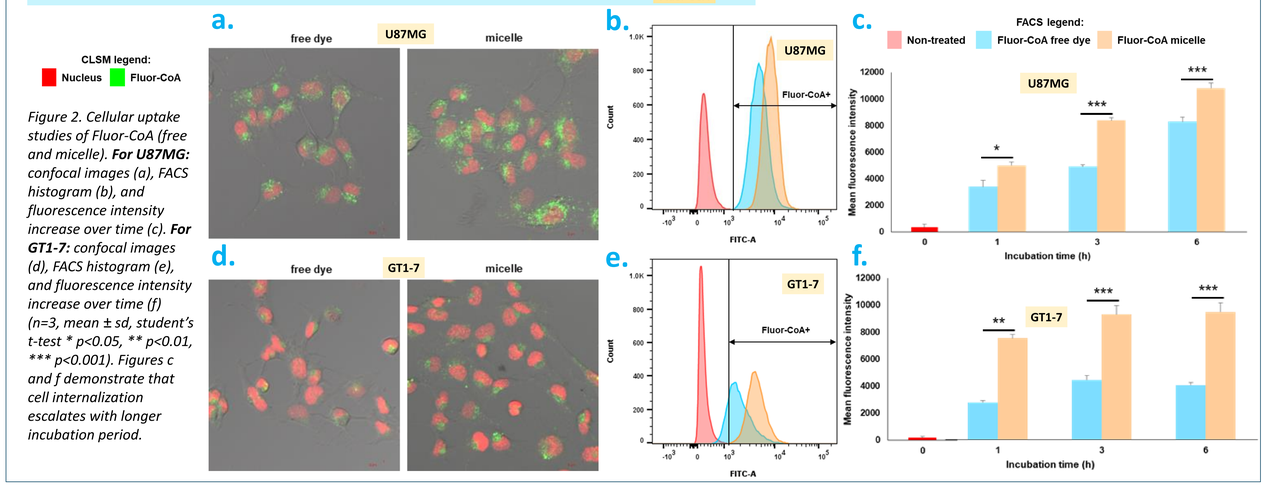


*Figure 2. Cellular uptake studies of Fluor-CoA (free and micelle). **For U87MG:** confocal images (a), FACS histogram (b), and fluorescence intensity increase over time (c). **For GT1-7:** confocal images (d), FACS histogram (e), and fluorescence intensity increase over time (f) (n=3, mean ± sd, student's t-test * p<0.05, ** p<0.01, *** p<0.001). Figures c and f demonstrate that cell internalization escalates with longer incubation period.*

CONCLUSION

Biological activity

(±)-, (+)-, and (-)-C75-CoA micelle **demonstrated significantly enhanced cytotoxicity against U87MG** and **lowered metabolic activity** over the free drug in both U87MG and GT1-7. CPT1 inhibition led to reduced FAO, as indicated by lower ATP production.

Cellular uptake

Fluor-CoA micelle showed **more efficient cellular uptake** in both U87MG and GT1-7, in comparison with the free dye. This corroborates the increased biological activity by C75-CoA micelle, because the micelle clearly enhances the intracellular concentration of CoA derivatives.

Overall

The cationic block co-polymer neutralized the negative charge of the CoA derivatives, resulting in a PIC micelle-type system that is effective in delivering small anionic molecules into selected brain cells. This strategy can be extended to deliver other anionic drugs for therapies in several diseases.

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